

Homework #4

Due 2/20/08

You are working on a drug that binds to a specific cellular protein. To learn more about the protein you would like to isolate the protein. As a first step you do a preliminary experiment to look at its solubility in $(\text{NH}_4)_2\text{SO}_4$.

Procedure: Cells are homogenized in the appropriate buffer and centrifuged for one hour at 100,000 x g. The pellet containing various membrane fractions is discarded and the supernatant is retained and labeled as 'cytosol'. One ml of the cytosol is placed into 5 tubes (labeled A-E) and the indicated amounts of $(\text{NH}_4)_2\text{SO}_4$ (in mg) are added to each tube. Following a one hour incubation on ice the samples are centrifuged and the supernatants discarded. The pellets are dissolved in one ml of buffer and then a portion of each sample (including the cytosol not subjected to $(\text{NH}_4)_2\text{SO}_4$ precipitation) was analyzed for drug-binding activity and for total protein as described below.

Drug-binding activity: 100 μl of each sample were mixed with the drug labeled with ^{14}C . Trichloroacetic acid (TCA) was added to each sample following a short incubation. TCA precipitates total protein including any bound drug. Drug not bound to protein remains soluble. The precipitated protein was collected on filters and unbound drug was washed away. In other words the cpm represent the drug-binding activity. Shown in the table are the cpm of radioactivity bound to protein. One μmole of drug is equal to 100,000 cpm. The blank contained all of the assay components (i.e., radioactive drug), but no protein, and was treated in a similar fashion. Examination of the channel ratios indicated that all of the samples had similar levels of quenching.

Total protein: The Bradford assay was used to determine the amount of protein in each sample. A standard curve with know amounts of protein (as indicated) was prepared and 10 μl of the various protein samples were assayed. Shown are the A_{600} values for the samples and the standards.

- For each of the samples calculate:
 - the percent saturated $(\text{NH}_4)_2\text{SO}_4$ (see nomogram in appendix of 'Differential Solubility' notes).
 - the drug-binding activity expressed as μmole drug bound per ml.
 - the protein concentration (mg/ml)
 - the specific activity (μmole drug bound per mg protein)
- Which sample (A-E) do you feel is best in terms of recovering drug-binding activity and at the same time reducing the total protein? Why?
- Briefly discuss ways in which the specific activity can be improved. In other words, how can you further optimize the $(\text{NH}_4)_2\text{SO}_4$ precipitation so that the maximum amount of drug-binding activity is recovered, but at the same time the total protein is reduced?

Results of measuring drug binding activity and protein content:

Sample	mg $(\text{NH}_4)_2\text{SO}_4$ added	cpm drug bound	A_{600}
Blank	-	103	-
Cytosol	-	10,482	0.765
A	114	319	0.107
B	209	1,497	0.254
C	313	8,708	0.409
D	430	10,165	0.549
E	561	9,748	0.689

Standards used in protein calculations:

Standards	A_{600}
2.5 μg	0.162
5.0 μg	0.257
10.0 μg	0.604
15.0 μg	0.801